

## THE EFFECT ON CALCULATED RESULTS OF ANALYSIS CAUSED BY THE VARIABILITY AMONG TOXAPHENE REFERENCE STANDARDS

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### Abstract

Toxaphene is a chlorinated camphene insecticide, containing more than 170 components, which yield a complex, multi-component gas chromatogram. The accurate identification and measurement of toxaphene in the environment depend on the availability of a suitable reference standard. To study the suitability of available standards, toxaphene standards were purchased from nine suppliers. The comparison of these standards using electron capture gas chromatography resulted in the discovery of a wide range in the relative proportions of the gas chromatography peaks among the materials studied. Such variations have serious effects on the qualitative and quantitative determination of toxaphene in environmental samples. Results of analysis show that the calculated values vary between 19% and 131% of the true concentration of solutions prepared in the laboratory. The use of total peak area under the envelope of toxaphene also demonstrates significant differences caused by the differences in the distribution of the component peaks. Because of these effects on the qualitative identification and quantitative determination of toxaphene, commercial suppliers and the regulatory agencies should work to establish a valid reference standard for the determination of toxaphene in environmental samples.

### Introduction

Toxaphene has been a widely used pesticide in the United States. Over the past several years, Hercules has collected environmental samples as part of monitoring activities and has submitted those samples to contract laboratories for the determination of toxaphene. Recommended methods<sup>1)</sup> specify that quantitation is done by using peaks in the latter half of the toxaphene chromatogram. Because Hercules observed differences among results of analyses between laboratories, a search for the cause of the variations was undertaken.

When no obvious differences (which could cause the observed variations in the results of analysis) in the practice of the methods were discovered, the question of the uniformity of toxaphene calibration standards was investigated. In the summer and fall of 1995, a survey by Hercules personnel revealed that, in the United States, there were more than twenty providers of toxaphene reference standards: but, upon further investigation, the number of primary suppliers was reduced to nine.

In a comparison of five toxaphene standards, Buser and Müller<sup>2)</sup> showed by GC-ECD and by GC-ECNIMS that the distribution of components differed significantly among the materials which were evaluated in their work on the characterization of two toxaphene components in lake sediment. Also, in a study of the composition of toxaphene detected in Lake Ontario sediment, Howdeshell and Hites<sup>3)</sup> observed differences in the homologue profiles among three toxaphene standards. Neither of those papers investigated the effects which those observed differences would have on the quantitative results calculated from the respective standards using approved EPA methods.

The data reported here will demonstrate the variability in the results of analyses calculated for different commercially available toxaphene reference standards.

### **Experimental Methods**

Samples of "neat" toxaphene were purchased from each supplier, and a solution of toxaphene in methanol was purchased from one of the suppliers. Solutions of each reference standard were prepared in hexane, and those solutions were analyzed according to EPA Method 8080<sup>1)</sup> using gas chromatography with an electron capture detector (GC-ECD). Sample components were separated on 30-meter X 0.53-mm J&W Megabore columns: DB-5 and DB-1701. Helium carrier gas flow was 10 mL/minute. The GC oven temperature profile was: 180°C; hold for 2 minutes; program to 240°C at 6°C/minute; hold at 240°C for 15 minutes. Flash injector temperature was 220°C, and the detector was maintained at 350°C. Hexane solutions (2 $\mu$ L) of all standards were injected into a Hewlett-Packard Model 5890 gas chromatograph, and the detector response was monitored by Hewlett-Packard Asterix ChemStation software. Component peak areas were measured electronically by two methods: 1.) valley-to-valley lines and 2.) baseline under the entire envelope of peaks in each chromatogram. The concentration of each reference material was calculated using a five-point calibration curve prepared from the reference sample, Hercules technical toxaphene X16189-49, which was produced in the Hercules Brunswick, Georgia, plant in 1968 (Lot No. 8BC25; 68.6 % Cl).

### **Results and Discussion**

Injection of the various commercially available toxaphene reference standards produced GC-ECD chromatograms which showed significant differences in the distribution of the component peaks of toxaphene. Hercules technical toxaphene X16189-49 was used as the reference standard for this work because it was a material whose origin was known. In fact, this same reference material was used in the work of Buser and Müller.<sup>2)</sup> While most of the samples contained component peaks with similar retention times, the relative proportions of those components varied widely among the samples. Those qualitative differences were reported in a poster<sup>4)</sup> at the EnvirACS 1996 exposition.

In the quantitative work reported here, the concentration ( $\mu$ g/mL) of each commercial reference material was calculated from a 5-point calibration curve based on Hercules technical toxaphene X16189-49. When matrix interferences are present, EPA Method 8080 recommends selecting peaks late in the chromatogram. Therefore, seven peaks were selected in the latter half of

the toxaphene chromatogram; and the corresponding peaks in each sample were used to determine total peak areas. The peak areas were calculated electronically using either a valley-to-valley baseline or a baseline constructed under the complete envelope of peaks in each sample. Integration parameters were adjusted so that the selection of baseline points was consistent between the reference standard and the other samples.

The results reported in Table 1 show that the distribution of the component peaks in toxaphene has a significant effect on the amount of toxaphene calculated in any sample. In the samples which most resemble the toxaphene X16189-49 reference standard, the agreement between the "Prepared Concentration" and the " $\mu\text{g/mL}$ , Calculated as Toxaphene" value is best. The reference standard from Crescent Chemical is the poorest match for toxaphene by comparison of both

Table 1. Comparison of Calculated Results for Different Toxaphene Standards\*

Suppliers of Toxaphene Reference Standard	$\mu\text{g/mL}$ , Calculated as Toxaphene				
	Prepared Concentration	DB-5 Column		DB-1701 Column	
		Valleys	Baseline	Valleys	Baseline
HERCULES	5.03	---	---	---	---
ACCUSTANDARD	3.80	3.71	3.73	3.82	3.61
EM SCIENCE	3.20	3.84	3.75	3.84	3.75
SUPELCO	4.65	6.02	4.20	5.48	4.95
POLYSCIENCE	4.83	3.30	3.75	3.21	3.25
RADIAN	5.16	6.32	5.03	5.61	5.60
PROTOCOL	4.86	4.26	5.22	3.90	4.09
CRESCENT	5.14	0.97	1.96	1.39	1.92
CHEM SERVICE	5.48	1.21	2.54	1.95	2.66
ULTRA SCIENTIFIC "Neat"	5.38	7.05	5.94	5.64	5.65
ULTRA SCIENTIFIC "Methanol Solution"	5.00	1.42	3.00	1.74	3.16

\* = Based on total areas of seven component peaks in the latter half of the chromatogram.

the GC-ECD chromatograms and the quantitative amount calculated for its solution. It is obvious from the data in Table 1 that the GC-ECD pattern of the reference standard will change the value calculated for toxaphene. For the reference materials studied here, the amount of toxaphene calculated will vary between 19% and 131% of the amount known to be present. Those variations make it very difficult to determine accurately not only the quantitative amount of toxaphene present in a sample but also the qualitative identification of a chromatogram as toxaphene.

Particularly troublesome is the variation between the two toxaphene reference standards purchased from Ultra Scientific. The "neat" standard material matches the Hercules technical toxaphene X16189-49 standard better than does the methanol solution; therefore, the quantitative amount calculated for the "neat" material is closer to the "Prepared Concentration." This situation illustrates the problem of obtaining consistent reference standards for toxaphene -- even from the same supplier.

Table 2. Comparison of Results of Analysis of Different Toxaphene Standards using Total Peak Area of All Components\*\*

Suppliers of Toxaphene Reference Standard	Calculated as Toxaphene				
	Prepared Concentration $\mu\text{g/mL}$	DB-5 Column		DB-1701 Column	
		Total Peak Area	$\mu\text{g/mL}$	Total Peak Area	$\mu\text{g/mL}$
HERCULES	5.03	59,403,540	---	27,607,155	---
ACCUSTANDARD	3.80	45,058,580	3.82	19,459,346	3.55
EM SCIENCE	3.20	46,600,896	3.95	19,937,278	3.63
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PROTOCOL	4.86	55,752,740	4.72	26,127,006	4.76
CRESCENT	5.14	44,368,520	3.76	19,428,369	3.54
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ULTRA SCIENTIFIC "Methanol Solution"	5.00	65,467,467	5.19	16,659,067	5.38

\*\* = Based on total areas of all component peaks in the chromatogram.

Because the selection of the seven peaks for quantitation of the toxaphene is subjective, the sample chromatograms were calculated using the total peak area under the entire envelope of peaks for each toxaphene reference material. The data in Table 2 show that there are still significant differences in the total GC-ECD response for each sample, and the concentration in  $\mu\text{g/mL}$  varies significantly among the samples. In general, the samples which gave the lowest calculated values in Table 1 also give the lowest values in Table 2, although the differences are not as large. The exception is the "Methanol Solution" from Ultra Scientific, which showed a significant increase in the concentration of toxaphene calculated using the total peak area procedure. This is an example of the effect of shifting the distribution of the components to the early eluting GC peaks. However, the use of the total area of all peaks is not the preferred method for measurement of toxaphene in environmental samples by standard EPA methodology using GC-ECD because of the potential for interference from matrix components and other chlorinated pesticides. While comparison of total peak areas is useful for demonstrating the differences in composition of the commercially available reference standards, it is not satisfactory for determining toxaphene in an environmental sample.

### Conclusions

This comparison of toxaphene reference standards from commercial suppliers has demonstrated significant variations among the materials currently available. These variations have serious implications for the accurate identification and quantitation of toxaphene in environmental samples.

It should be noted that it has been almost seventeen years since toxaphene was manufactured in the United States. "Toxaphene" made outside the United States may or may not have used the same processes used by Hercules and other U.S. manufacturers. There is no information available that compares the gas chromatograms of these various "toxaphenes" using modern capillary columns and detectors. Consequently, differences in manufacturers and/or processes might account for the differences in the purchased standards.

Action should be taken by the suppliers of reference standards, or by the regulatory agencies, to establish a valid toxaphene reference standard for use by all laboratories involved in the analysis of environmental samples for toxaphene.

### Acknowledgments

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### Literature Cited

- 1.) U.S. Environmental Protection Agency, "Test Methods for Evaluation of Solid Waste, Volume 1B: Laboratory Manual, Physical/Chemical Methods, (SW-846)," Method 8080A, Organochlorine Pesticides and Polychlorinated Biphenyls by Gas Chromatography, Revision 1, November 1992.
- 2.) Buser, H.R.; Müller M.D. *J. Agric. Food Chem.* **1994**, 42, 393-400.
- 3.) Howdeshell, M.J.; Hites, R.A.; *Env. Sci. Tech.* **1996**, 30 (1), 220-224.
- 4.) Carlin, Jr., F.J.; Hoffman, J.M. Proceedings of the Twelfth Annual Waste Testing and Quality Assurance Symposium, Washington, D.C., July 23-26, 1996, 148-155.

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## OVERVIEW

Toxaphene contains more than 170 components.

Multicomponent electron capture gas chromatogram

Toxaphene reference standards purchased from nine suppliers compared on two different GC columns

Significant differences in electron capture GC profiles affected quantitative determination.

Calculated values vary between 19% and 131% of the true concentration of solutions prepared in the laboratory.

Reliable standards needed for identification and quantitation



## INTRODUCTION

Toxaphene has been a widely used pesticide in the United States. Over the past several years, Hercules has collected environmental samples as part of monitoring activities and has submitted those samples to contract laboratories for the determination of toxaphene. U.S. EPA-approved methods <sup>1)</sup> specify that quantitation is done by using peaks in the latter half of the toxaphene chromatogram. Because Hercules observed differences among results of analyses between laboratories, a search for the cause of the variations was undertaken.

When no obvious differences (which could cause the observed variations in the results of analysis) in the practice of the methods were discovered, the question of the uniformity of toxaphene calibration standards was investigated. In the summer and fall of 1995, a survey by Hercules personnel revealed that, in the United States, there were more than twenty providers of toxaphene reference standards; but, the number of primary suppliers was reduced to nine.

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The data reported here will demonstrate the variability in the results of analyses calculated for different commercially available toxaphene reference standards.

## METHODS

Instrument: HP 5890 GC with Ni<sup>63</sup> electron capture detector

GC Columns: 30-meter x 0.53-mm ID; J&W Megabore:  
a.) DB-1701                      b.) DB-5

Temperature: 180° C; hold for 2 minutes; program to 240° C at 6° C/minute; hold at 240° C for 15 minutes.

Data System: HP Asterix ChemStation

Peak Areas: Measured: 1.) valley-to valley and 2.) baseline under all peaks

Samples: Toxaphene reference standards dissolved in hexane

Calibration: 5-point, using Hercules technical toxaphene X16189-49

Samples of "neat" toxaphene were purchased from each supplier, and a solution of toxaphene in methanol was purchased from one of the suppliers. Solutions of each reference standard were prepared in hexane, and those solutions were analyzed according to EPA Method 8080<sup>1)</sup> using gas chromatography with an electron capture detector (GC-ECD). Hexane solutions (2 $\mu$ L) of all standards were injected into a Hewlett-Packard Model 5890 gas chromatograph, and the detector response was monitored by Hewlett-Packard Asterix ChemStation software.

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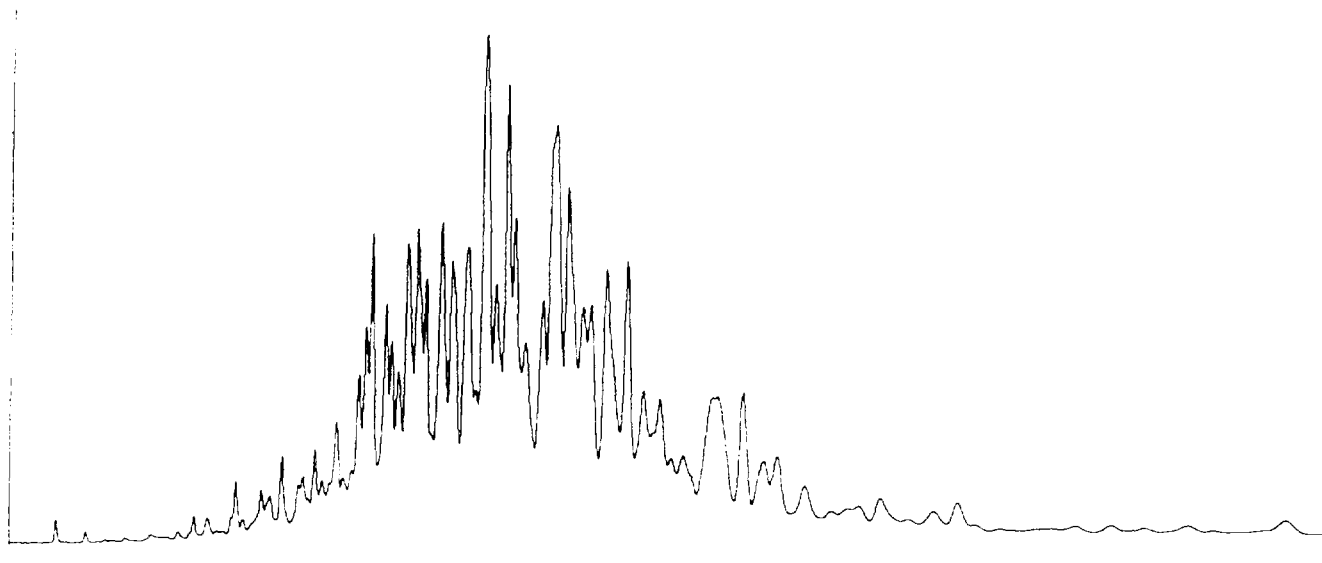
# RESULTS

FIGURE 1.

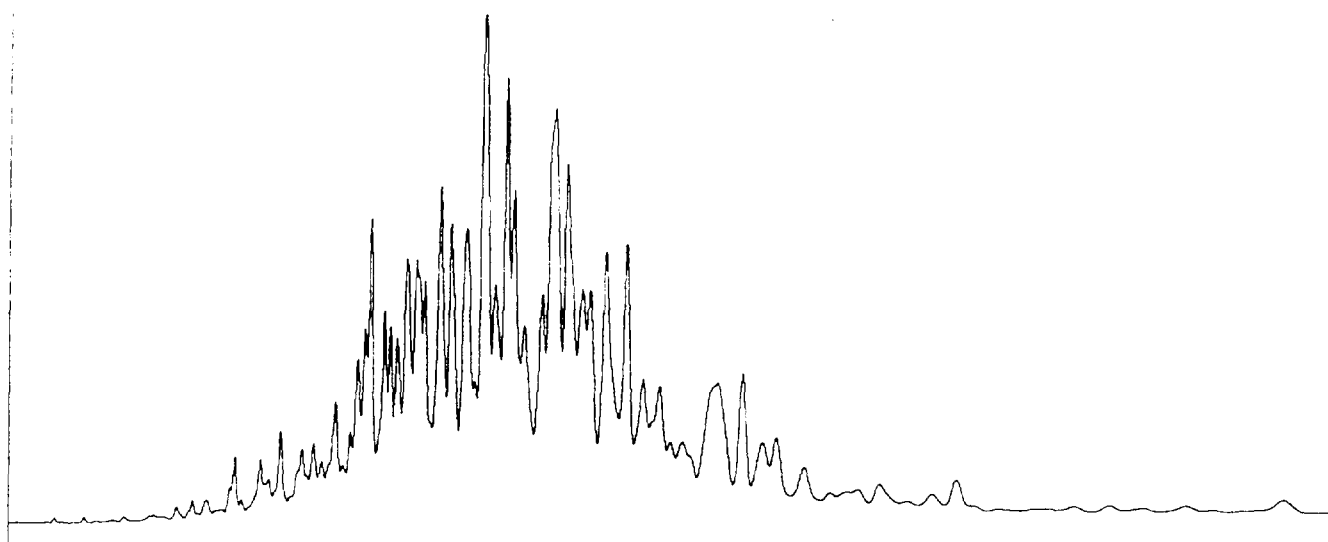
COMPARISON OF TOXAPHENE STANDARDS

1. 7 0014

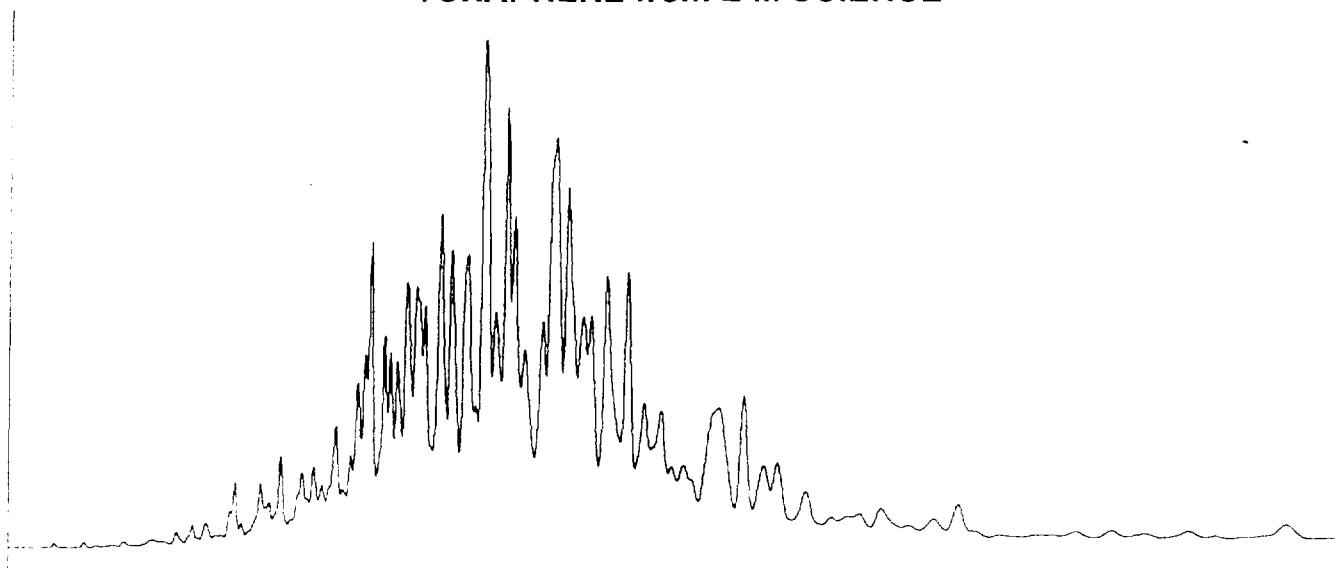
HERCULES TECHNICAL TOXAPHENE A16189-49



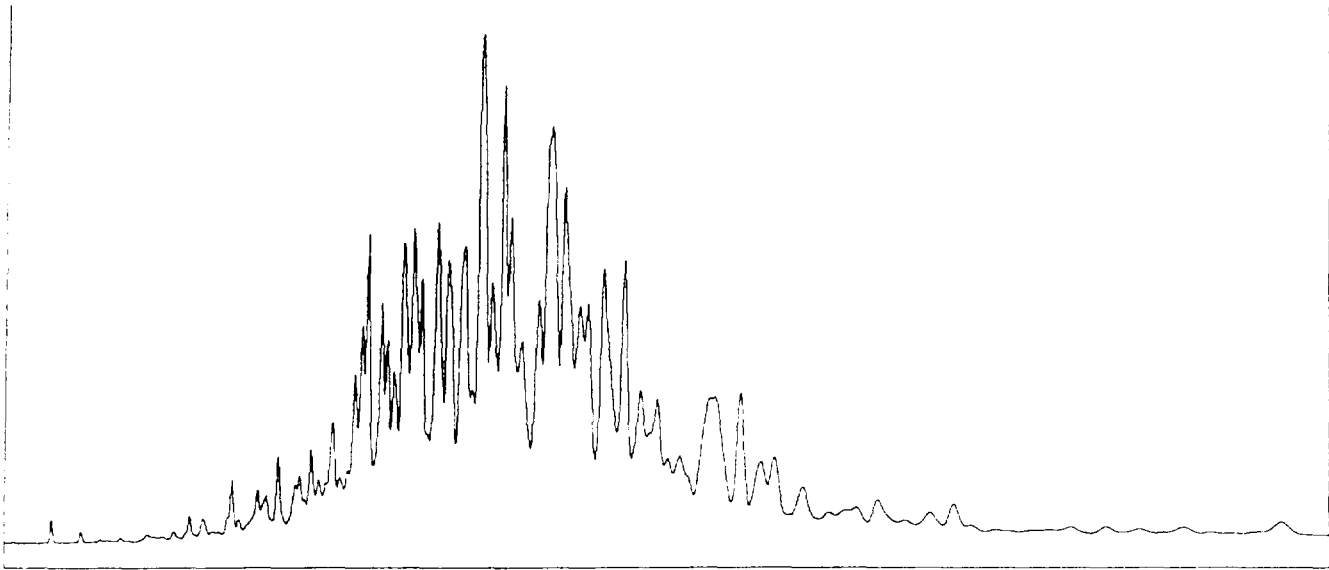
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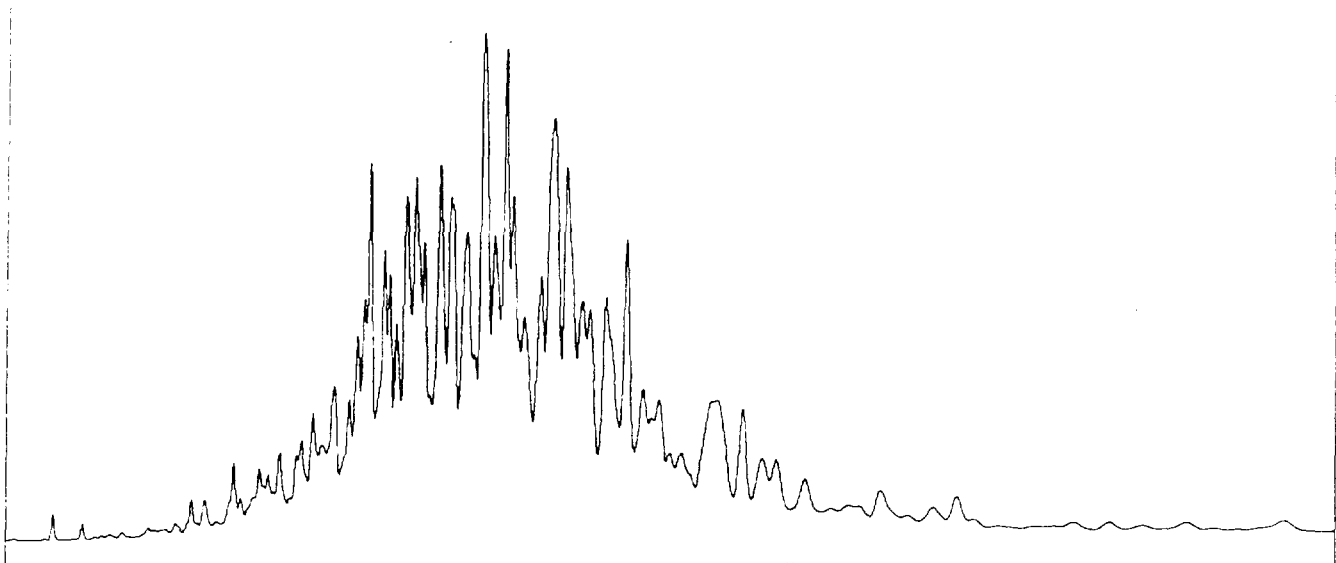
TOXAPHENE from E M SCIENCE



## HERCULES TECHNICAL TOXAPHENE A16189-49



## TOXAPHENE from POLYSCIENCE



## TOXAPHENE from RADIAN

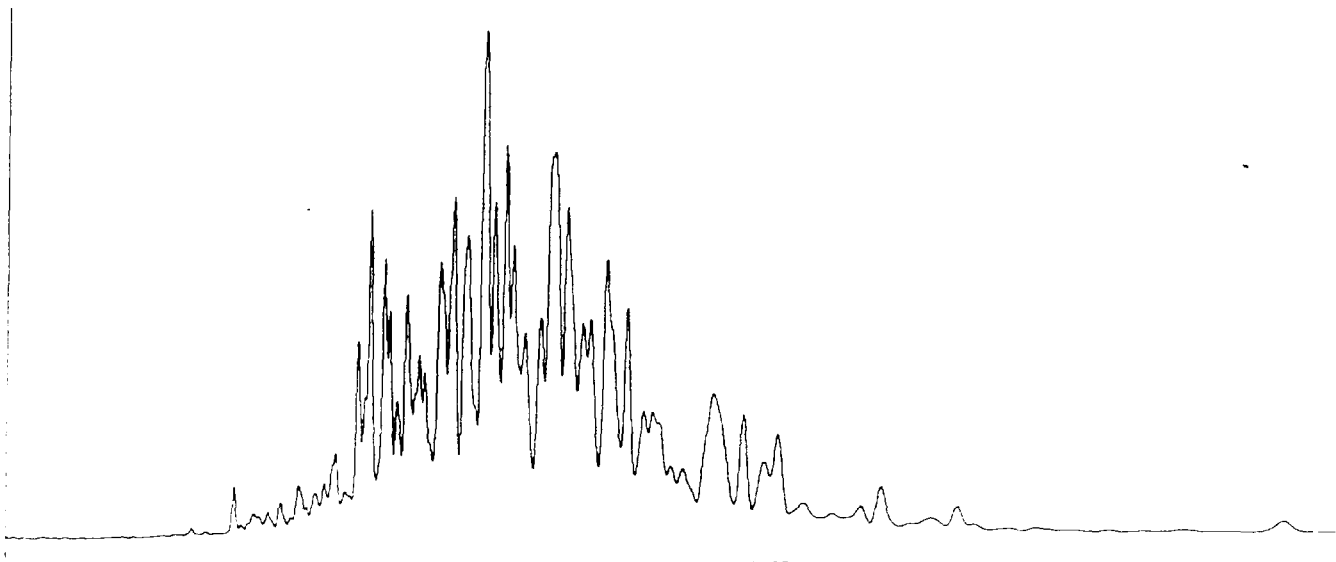
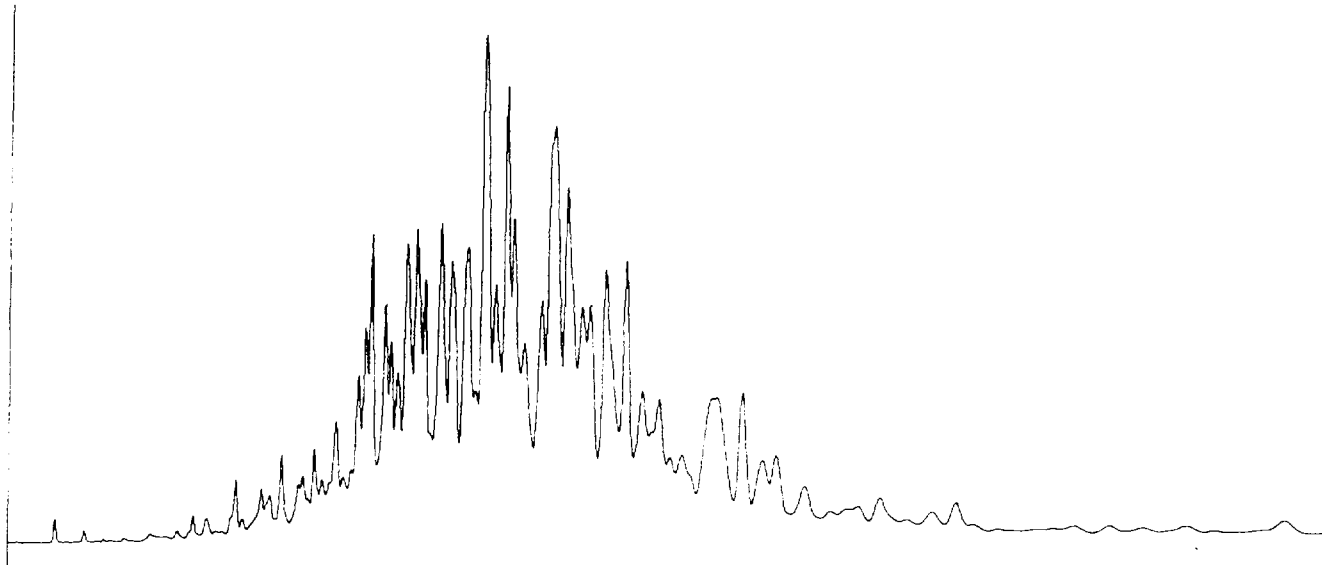


FIGURE 3.

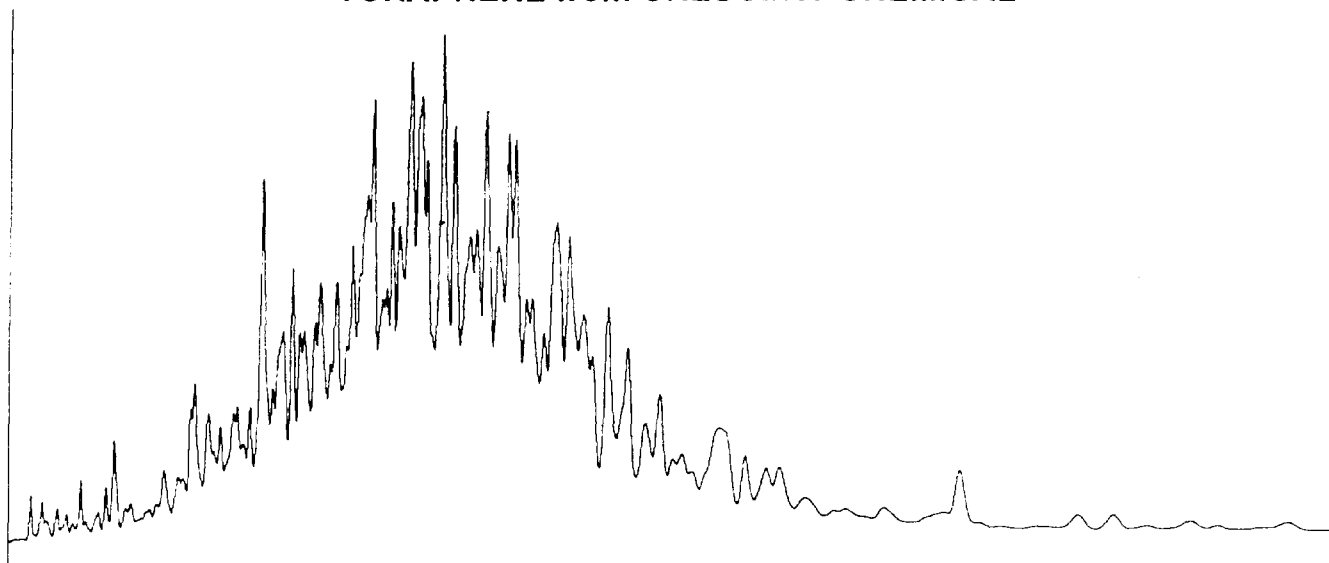
COMPARISON OF TOXAPHENE STANDARDS

11 7 0016

HERCULES TECHNICAL TOXAPHENE  $\lambda$ 16189-49



TOXAPHENE from CRESCENT CHEMICAL



TOXAPHENE from ULTRA SCIENTIFIC

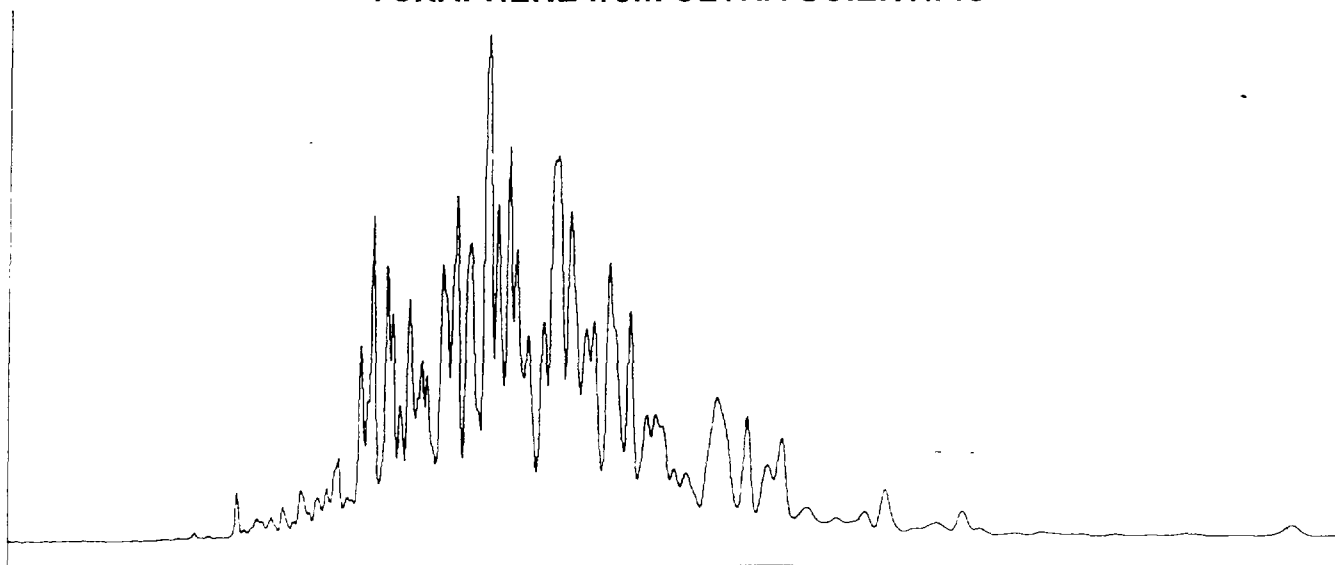
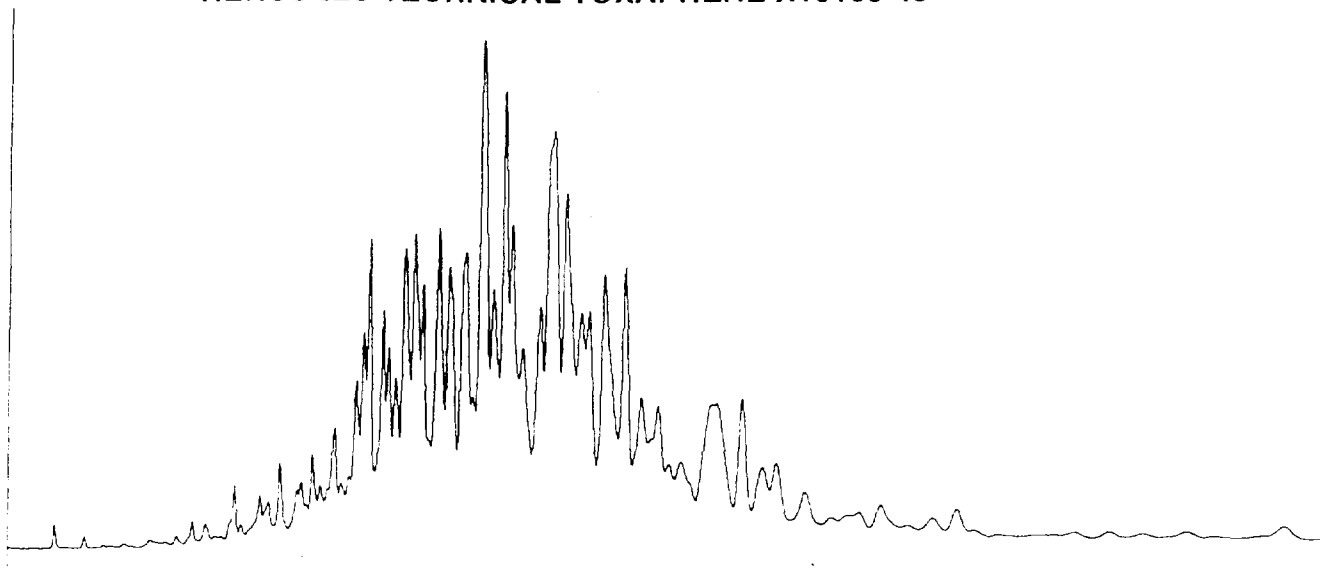




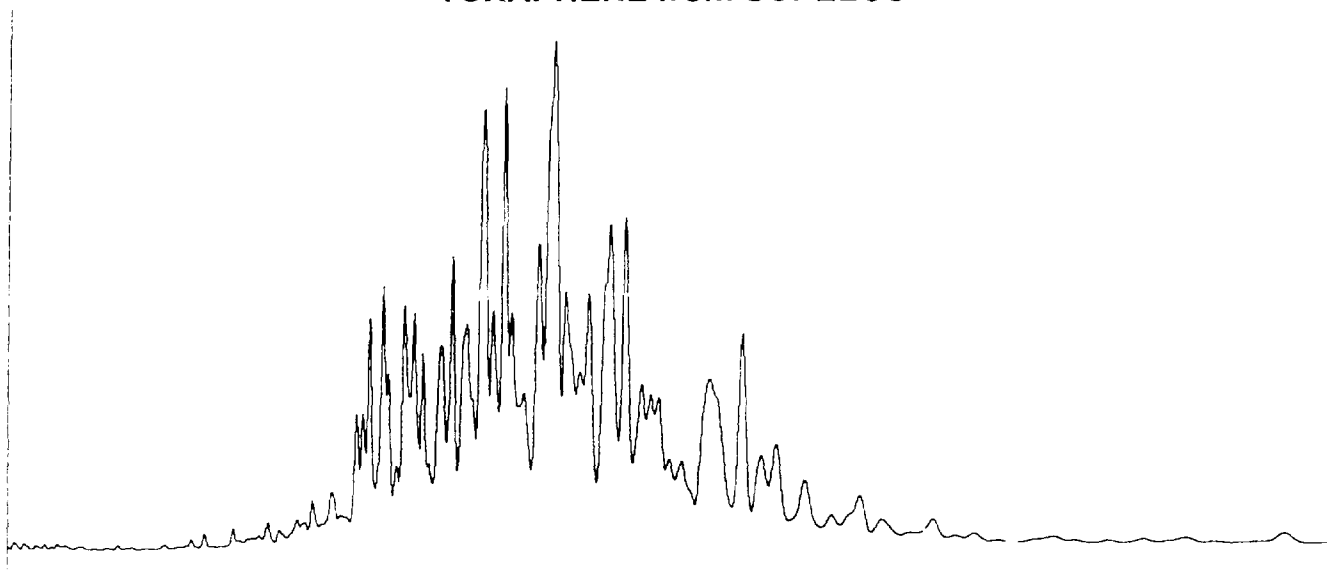
FIGURE 4. COMPARISON OF TOXAPHENE STANDARDS

HERCULES TECHNICAL TOXAPHENE X16189-45

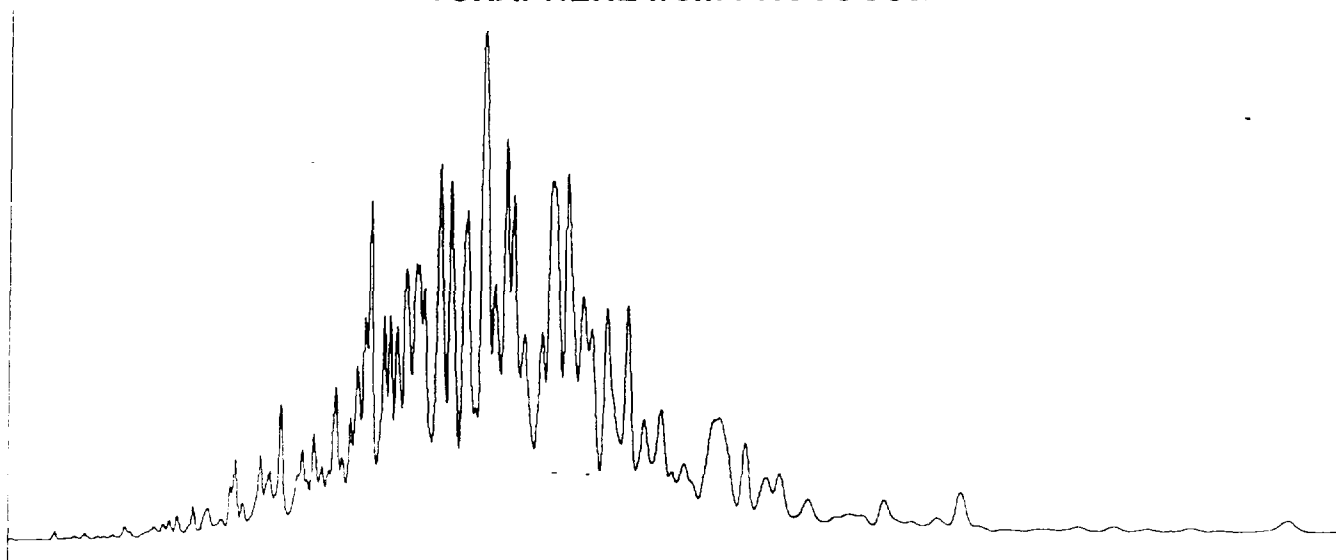
17 7 0017

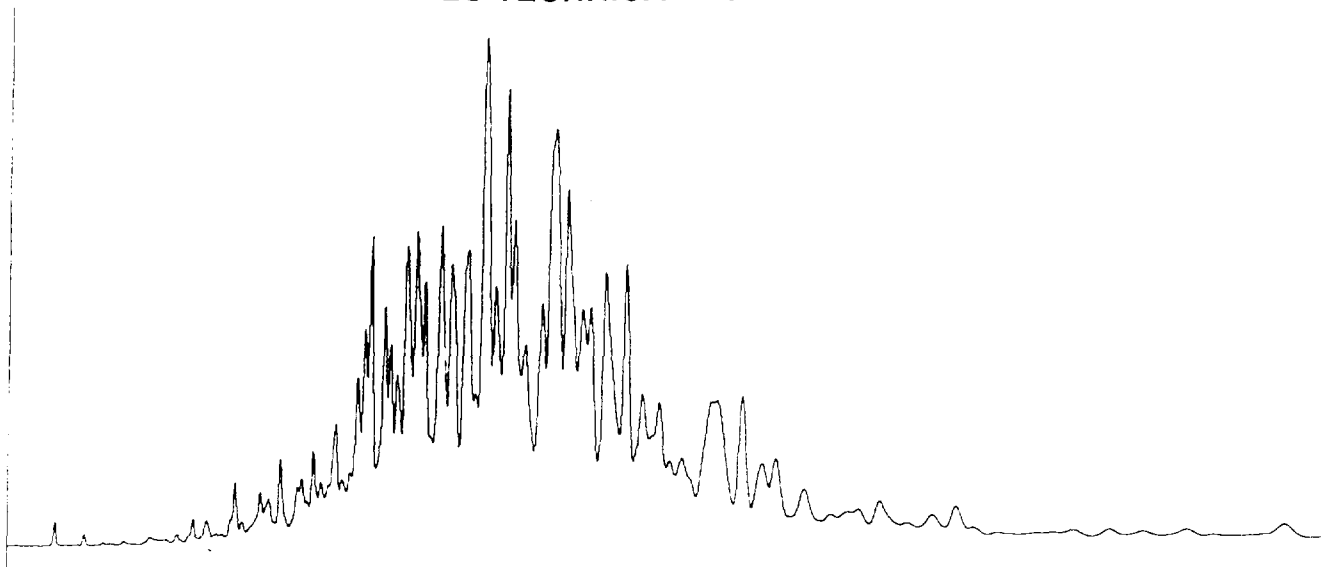


TOXAPHENE from SUPELCO

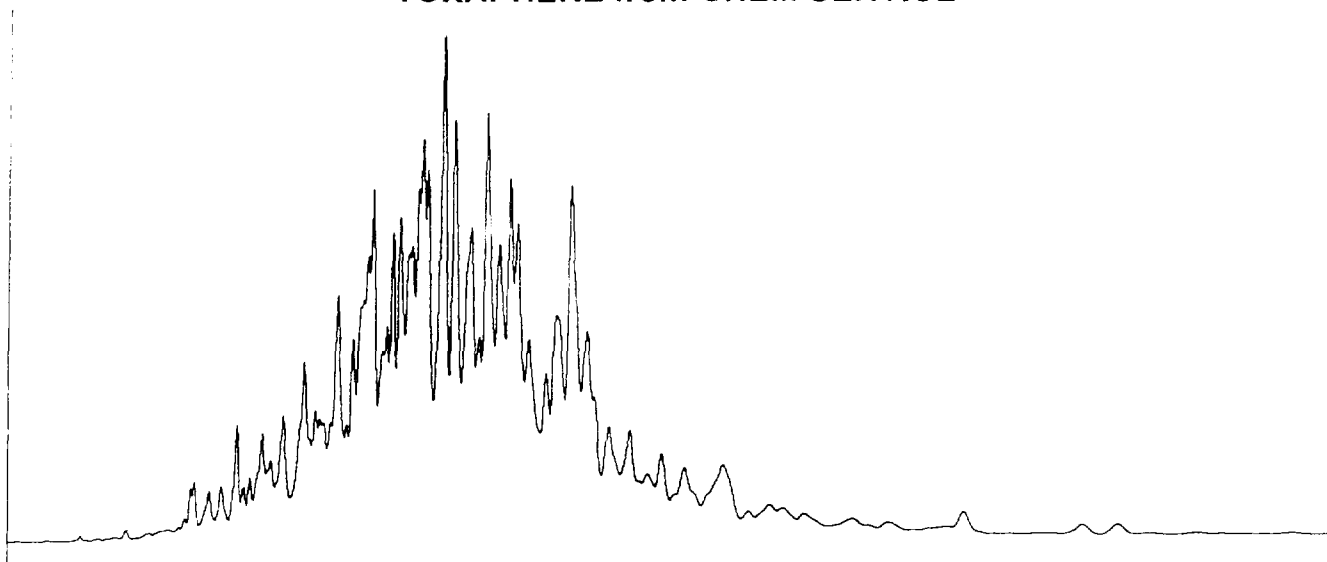


TOXAPHENE from PROTOCOL



HERCULES TECHNICAL TOXAPHENE  $\lambda$ 16189-49

## TOXAPHENE from CHEM SERVICE



## TOXAPHENE from RESTEK

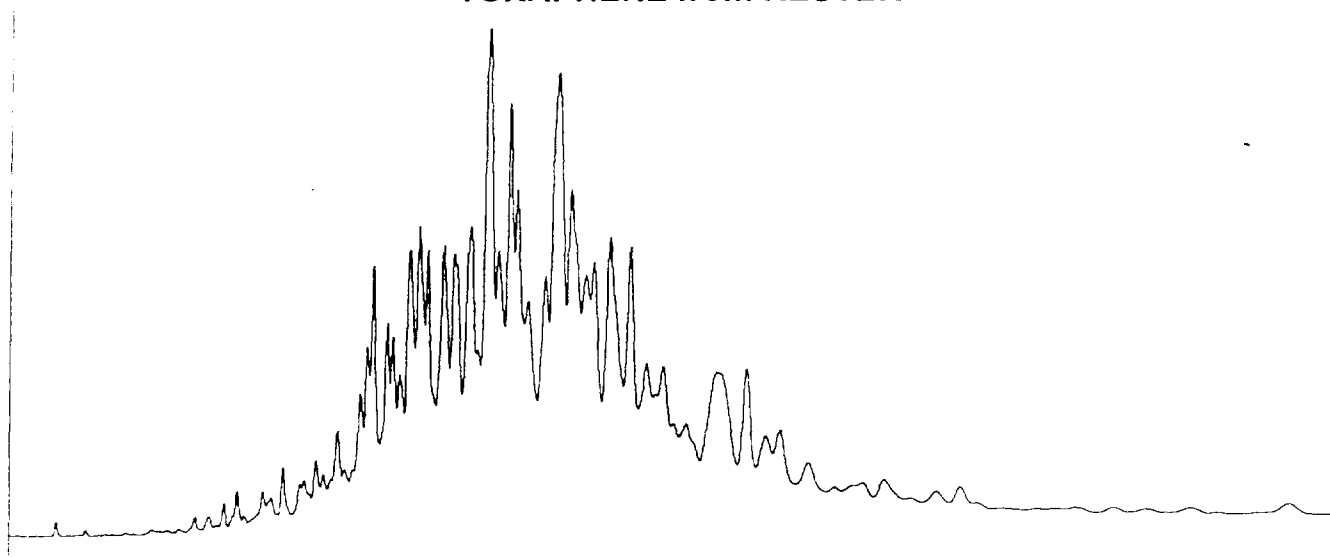
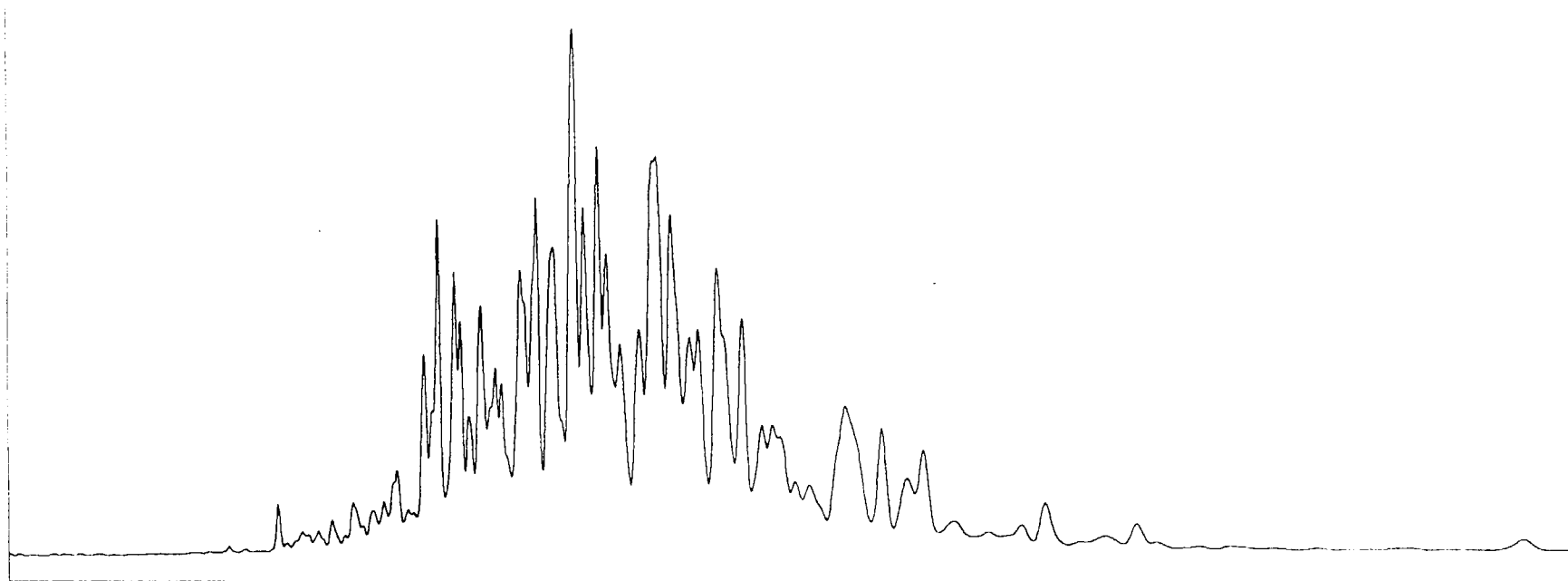


FIGURE 6.

COMPARISON OF TOXAPHENE STANDARDS FROM ONE SUPPLIER

ULTRA SCIENTIFIC "NEAT"



ULTRA SCIENTIFIC "METHANOL SOLUTION"

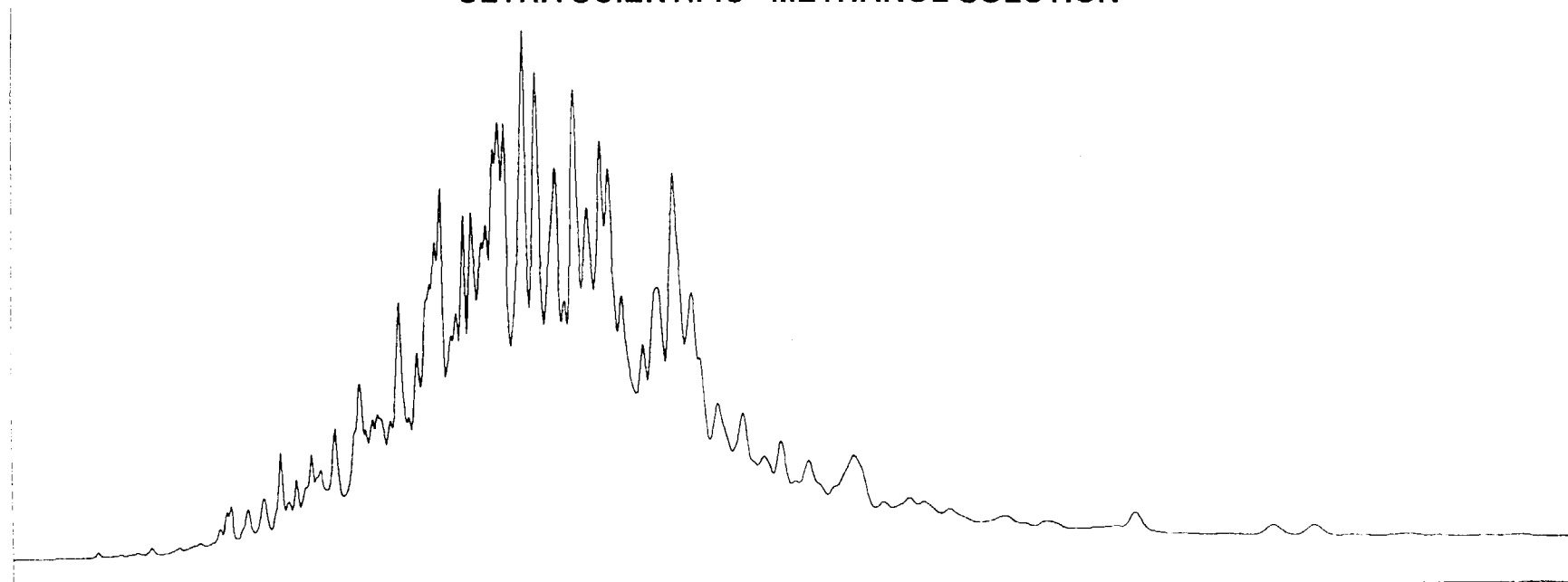


Table 7. Comparison of Calculated Results for Different Toxaphene Standards\*

Supplier of Toxaphene Reference Standard	Prepared $\mu\text{g/mL}$	$\mu\text{g/mL}$ , Calculated as Toxaphene			
		DB-5 Column		DB-1701 Column	
		Valleys	Baseline	Valleys	Baseline
HERCULES	5.03	---	---	---	---
ACCUSTANDARD	3.80	3.71	3.73	3.82	3.61
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## CONCLUSIONS

This comparison of toxaphene reference standards from commercial suppliers has demonstrated significant variations among the materials currently available. Two toxaphene standards purchased from the same supplier have shown significant differences. These variations have serious implications for the accurate identification and quantitation of toxaphene in environmental samples.

It should be noted that it has been almost seventeen years since toxaphene was manufactured in the United States. "Toxaphene" made outside the United States may or may not have used the same processes used by Hercules and other U.S. manufacturers. There is no information available that compares the gas chromatograms of these various "toxaphenes" using modern capillary columns and detectors. Consequently, differences in manufacturers and/or processes might account for the differences in the purchased standards.

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## LITERATURE CITED

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- 2.) Buser, H.R.; Müller M.D. *J. Agric. Food Chem.* **1994**, 42, 393-400.
- 3.) Howdeshell, M.J.; Hites, R.A.; *Env. Sci. Tech.* **1996**, 30 (1), 220-224.
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